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Review

P58^{IPK}, a novel cochaperone containing tetratricopeptide repeats and a J-domain with oncogenic potential

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Abstract. Tetratricopeptide repeats (TPRs) are loosely conserved 34-amino acid sequence motifs that have been shown to function as scaffolding structures to mediate protein-protein interactions. TPRs have been identified in a number of proteins with diverse functions and cellular locations. Recent studies suggest that individual TPR motifs can confer specificity in promoting homotypic and/or heterotypic interactions, often in a mutually exclusive manner. These features are best exemplified by the P58^{IPK} protein, an influenza virus-activated cellular inhibitor of the PKR protein kinase, whose different TPR motifs mediate interactions with distinct proteins. P58^{IPK}, which possesses cochaperone and oncogenic properties, represents a unique class of TPR proteins containing a J-domain. Here we review recent progress on the structural and functional characterization of P58^{IPK}, and discuss the possible mechanisms by which P58^{IPK} modulates PKR and induces tumorigenesis in view of present knowledge of TPR proteins and molecular chaperones.

Key words. Tetratricopeptide repeat; J-domain; P58^{IPK}; PKR; protein-protein interactions; cochaperone; protein folding; tumorigenesis.

Introduction

Many proteins in biological processes function as members of large complex assemblies, potentially comprising hundreds of copies of several different proteins. This includes the cytoskeletal proteins, nuclear pores, ribosomes, DNA transcription complexes and many signaling enzymes, to name a few. In addition to protein conformational switching events, an ordered sequence of specific protein-protein recognition plays a pivotal role in macromolecular assembly processes. Many interacting sites, including SH2, SH3, PDZ, PTB, PH, WW and C2 domains, have already been identified. Furthermore, additional protein components, such as chaperones or scaffolding proteins, are often required for correct protein folding and assembly, and function as a higher-order level of biological catalysis. Tetratricopeptide repeats (TPRs) are a relatively new class of interacting domains that are present in a number of proteins with diverse functions and subcellular locations. More recently, a subclass of TPR proteins, namely those that also contain a homology region to DnaJ family of chaperones, have emerged. This review will focus on one such member, the P58^{IPK} protein, showcasing the evolutionary selection of a molecule with multiple and different functional domains to allow communication between separate molecular pathways.

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Tetratricopeptide repeats: a new class of scaffolding structures?

TPR-containing proteins are an emerging family of proteins that are characterized by semiconserved 34-amino acid repetitive segments (for recent reviews, see [1, 2]). First identified in the cell division cycle genes *CDC16*, *CDC23* and *CDC27* of *Saccharomyces cerevisiae* in 1990, TPR motifs were initially predicted to form a 'knob and hole' structure consisting of two amphipathic α-helices separated by a spacer region [3, 4]. Such a configuration, known as 'snap helix', was proposed to mediate protein-protein interactions between TPR-containing proteins through the 'knob' of a TPR unit interlocking with the 'hole' of a different TPR unit. Furthermore, both helices in a single TPR unit were viewed as a linear arrangement with adjacent TPR units being arranged in an antiparallel manner. In contrast, when the crystallographic structure of the TPR domain of protein phosphatase 5 (PP5) was solved in 1998, each of the three TPR motifs of PP5 was found to adopt a pair of antiparallel α-helices [5]. Furthermore, contiguous TPR units were organized into a parallel orientation, resulting in a higher-order helical shape with an amphipathic groove. However, because of the sequence variability among TPR motifs, it would be premature to assume that all TPRs share the same three-dimensional configurations as those of PP5. Clearly, this issue will be resolved with the determination of the three-dimensional structures of additional TPR-containing proteins, including those in complex with different TPR proteins.

Proteins containing as few as 1 to as many as 16 TPRs have been identified, with multiple repeats being arranged in tandem and usually grouped into clusters or long stretches. TPR-containing proteins have been identified in virtually all types of cells, from bacteria to humans, with no apparent preference in cellular location. They are implicated in a variety of cellular processes, ranging from cell cycle control to signal transduction, and to kinase regulation. This is perhaps not surprising given the pivotal role of molecular interactions in biological processes; TPR motifs appear to recognize both TPR and non-TPR sequences. The former feature was first described in the *S. cerevisiae* cell division cycle proteins CDC16, CDC23 and CDC27. These proteins contain 10, 9, and 10 TPR motifs, respectively, with each protein being capable of interacting with itself and with one another [4, 6]. Deletion analysis demonstrated that the homo- and heterotypic interactions among these cell cycle proteins are mediated by TPRs. The notion that TPR motifs may also bind non-TPR-containing proteins was demonstrated by another yeast TPR protein, Cyc8 (also called Ssn6), which binds to the transcriptional corepressor Tup1 [7]. Subsequent studies of several other TPR-containing proteins also show that they are involved in intermolecular interactions with different TPR proteins, as well as with non-TPR proteins. A list of TPR-containing proteins and their known associated partners is shown in table 1. For a more complete list of TPR-containing proteins, the readers are referred to the Protein Families Database of Alignments and Hidden Markov Models (http://www.sanger.ac.uk/Software/Pfam/). It should be stressed, however, that in some of these studies only the domain that contains the TPR unit(s) was examined. Thus, the possibility that flanking sequences may also play a role in the interactions cannot be excluded.

An area of research that has contributed important insights into TPR-mediated protein-protein interactions is the glucocorticoid receptor-mediated signaling pathway. Steroid receptor complexes contain several TPR proteins that interact primarily with the molecular chaperones Hsp70 and Hsp90 (reviewed in [8]). The cyclophilin Cyp40 and immunophilins FKBP51 and FKBP52 each contain three TPR motifs, and all interact with Hsp90 [9]. The cochaperone Hop (also called Sti1 in yeast) is found to associate with both Hsp70 and Hsp90 through its TPR motifs [10]. Similarly, the PP5 protein phosphatase is a component of glucocorticoid receptor heterocomplexes that associates with Hsp90 through its TPR domain [11]. Studies of the Hsp90-interactive TPR proteins suggest that there may be a general 'TPR recognition motif', as these proteins compete with one another for interaction with the chaperone [12]. However, this is not the whole story for every TPR-binding protein. There may be a consensus sequence motif for TPR binding, but the specificity of protein-protein recognition may also be influenced by the specific composition of nonconsensus residues and flanking domains. This in fact appears to be the case with TPR proteins Cyp40 [12], Hip [13] and CHIP [14]. Alternatively, the specificity is dictated by the TPR motifs themselves, dependent on the three-dimensional structural arrangement conferred by adjacent TPR units. Both predictions will likely turn out to be true if TPRs are to achieve diverse and specific molecular interactions. Indeed, the TPR protein Hip binds noncompetitively to Hsp70 in the presence of Hop [15]. Thus, Hsp70 appears to have two TPR-binding sites, which are recognized by different TPR proteins.

The protein kinase inhibitor P58^{IPK}: TPR motifs find a model system in the interferon-induced antiviral response pathway

One of the best-characterized TPR proteins is P58^{IPK}, which associates with different proteins through distinct TPR domains. The P58^{IPK} molecule contains nine TPR

motifs, arranged in tandem, which comprise over 60% of the P58^{IPK} sequence (fig. 1). In addition, the C-terminus of P58^{IPK} contains a region homologous to the J-domain of DnaJ family of proteins. The 58-kDa P58^{IPK} (inhibitor of protein kinase) protein was originally identified as an inhibitor of the interferon (IFN)-induced serine/threonine protein kinase PKR [16, 17]. Thus, we will first provide a brief review of PKR.

PKR (protein kinase, RNA-activated) is an important mediator of the cellular IFN-induced antiviral response, at least in part, through its ability to repress protein synthesis by phosphorylating the eukaryotic initiation factor 2, on the α subunit (eIF2α) (reviewed in [18]). Although PKR is induced by IFN, the enzyme is thought to be normally latent. PKR is activated upon binding double-stranded RNA (dsRNA) or highly structured RNA molecules, a process that is associated with kinase dimerization and autophosphorylation. Viral RNAs are potent activators of PKR, as they are produced at high levels during an acute infection, and frequently contain ds structural elements. In addition, many viruses synthesize dsRNA as part of their replicative process. Once activated, PKR catalyzes the phosphorylation of eIF2α on serine 51 (S51). eIF2α is a critical component for translation initiation, and is inactivated by phosphorylation at S51, leading to an inhibition of translation initiation. Thus, PKR shuts down protein synthesis in a virally infected cell to prevent or limit the spread of virus infection.

The increasing number of different viruses that use distinct mechanisms to downregulate PKR attests to the importance of the protein kinase as part of the antiviral response [18, 19]. For example, vaccinia virus and reovirus utilize viral dsRNA-binding proteins (E3L and σ3, respectively) to prevent PKR activation by sequestering potential activator dsRNAs [20, 21]. Adenovirus inhibits PKR by encoding a short, structured RNA molecule (VAI RNA) that binds to PKR and prevents interaction with *bona fide* activator RNA molecules [22]. Some viruses, such as vaccinia virus [23] and HIV-1 [24], encode eIF2α-like proteins that act as pseudosubstrate inhibitors of PKR. Recent work from our

Table 1. A partial list of TPR proteins and their known interacting partners [88].

Organism/protein	Binding partner(s)	Self-associates?	Function/pathway	Reference
Saccharomyces cerevisiae				
CDC16	CDC27, CDC23	yes	cell cycle	6
CDC23	CDC16, CDC27	yes	cell cycle	6
CDC27	CDC16, CDC23	yes	cell cycle	6
CNS1	Hsp82, Cpr7	?	Hsp90 cochaperone	69
Cpr6	Hsp82, Rpd3	?	Hsp90 cochaperone	70, 71
Cpr7	Hsp82, Rpd3	?	Hsp90 cochaperone	70, 71
Cyc8 (SSN6)	Tup1	?	transcription repression	7
Cyp40	Hsp90	?	Hsp90 cochaperone	72
PEX5	peroxisome targeting signal (PTS)	?	peroxisome signaling	73
Prp39p	U1 SnRNP, Mud2p	?	mRNA splicing	74, 75
Prp42p	U1 SnRNP	?	mRNA splicing	76
Sti1	Hsp90	?	Hsp90 cochaperone	77
Pichia pastoris				
PEX5	PTS1	?	peroxisome assembly	78
Aspergillis nidulans				
BIMA	MPM2	?	cell cycle	79
Caenorhabditis elegans				
SMG-7	SMG-5	?	mRNA surveillance	80
Drosophila melonagaster				
Kinesin light chain	cellular cargo proteins	?	kinesin regulation	81
Plants				
Wheat FKBP	Hsp90	?	Hsp90 cochaperone	82
Xenopus laevis				
PP5	CDC16, CDC27	?	anaphase-promoting complex	83
Mammals				
CHIP	Hsc70	?	cochaperone	84
Cyp40	Hsp90	?	cochaperone	82
FKBP51	Hsp90	?	cochaperone	85
FKBP52	Hsp90	?	cochaperone	82, 86
Hip	Hsc70	yes	cochaperone	87, 88
Hop	Hsp70, Hsp90	?	cochaperone	10
P58^{IPK}	Hsp40, Hsp70, P52^{rIPK}, PKR	yes	cochaperone, PKR inhibitor	26–29
PP5	Hsp90	?	phosphatase	11
Tpr1	neurofibromin	?	?	35
Tpr2	neurofibromin	?	?	35

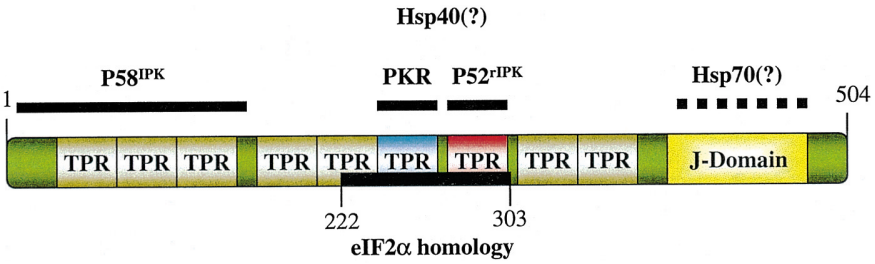

Figure 1. Schematic diagram of the primary structures of P58IPK. Tetratricopeptide repeats (TPR-labeled boxes) and J-domain (yellow box), as well as a central region (black bar) of homology to eIF2α, the natural substrate of PKR, is indicated. The regions of interaction with P58IPK-interacting molecules, PKR and P52rIPK, are indicated in top bars. Also indicated is the P58IPK self-interaction region. See text for further details.

laboratory has shown that hepatitis C virus also encodes an inhibitor of PKR, NS5A, which binds to PKR and blocks dimerization [25, 26]. Some viruses even utilize host cell mechanisms to downregulate PKR activity. For example, poliovirus infection induces the degradation of PKR by an unidentified cellular protease [27], whereas influenza virus activates the cellular inhibitor P58IPK [16, 17], the subject of this treatise.

Structure and function of P58IPK

P58IPK is normally inactive due to its association with its own inhibitor(s). A negative regulator of P58IPK was discovered in a yeast two-hybrid screen for P58IPK-interacting proteins [28]. This protein, termed P52rIPK (repressor of inhibitor of protein kinase), is a novel protein that has a region sharing a limited homology to the charged domain of Hsp90. P52rIPK inhibits P58IPK through direct interaction, although we do not yet understand how the P52rIPK-P58IPK interaction is regulated in mammalian cells.

P58IPK is a typical TPR protein in that it interacts with heterologous proteins and self-associates [29–31]. Our laboratory has explored the role of TPR domains in P58IPK and has identified several regions of functional significance. The most important region, with respect to P58IPK function, is TPR6, which directly interacts with PKR (fig. 1) [31]. Indeed, a P58IPK variant protein that lacks TPR6 (P58IPKΔTPR6) is unable to inhibit PKR either in vitro or in vivo [17, 31]. The seventh TPR motif (TPR7) is required for the interaction with the P58IPK inhibitory protein, P52rIPK [28]. Moreover, functional analysis in yeast showed that a TPR7 deletion mutant (P58IPKΔTPR7) was a more effective inhibitor of PKR than wild-type P58IPK [28]. This suggests that P58IPKΔTPR7 lacks a negative regulatory domain, consistent with the role for P52rIPK as a negative regulator of P58IPK in vivo. However, the specific residues within TPR6 and TPR7, which directly contact PKR and P52rIPK, respectively, have not been identified.

Using a biochemical purification scheme based on PKR activity, our laboratory has also identified the eukaryotic DnaJ homologue Hsp40 as a negative regulator of P58IPK [29]. Influenza virus probably activates P58IPK by promoting the dissociation of Hsp40 from P58IPK during infection [29]. P58IPK and Hsp40 interact with each other in a direct manner, as determined with the use of purified proteins [29, 30]. In addition to Hsp40, P58IPK also forms a complex with the molecular chaperone Hsp70. However, the complex formation required both Hsp40 and ATP. The dependence on Hsp40 was confirmed in vivo by means of a yeast two-hybrid approach that measured P58IPK and Hsp70 interaction in the presence or absence of Hsp40. Furthermore, the binding was localized to the ATPase domain of Hsp70. It is not clear, however, whether Hsp40 promotes complex formation through its interaction with P58IPK (via protein conformational change) or acts as a molecular bridge between P58IPK and Hsp70. We favor the former possibility for reasons discussed below. Nor do we know which regions of P58IPK associate with the molecular chaperones. While there is no preliminary indication as to where Hsp40 may bind to P58IPK, Hsp70 could potentially interact with the TPR motifs and/or the J-domain of P58IPK, as in the case with other systems [33–35]. In this regard, it is noteworthy that removal of TPR5 was found to result in an increase in P58IPK activity [32], suggesting that a negative regulator, such as Hsp40, may bind to this site.

Using the yeast two-hybrid approach, the region of P58IPK that is required for self-interaction has been mapped to the N-terminal 166 amino acids, which contain TPR motifs 1–3 and part of TPR4 [31]. Interestingly, sequence comparison against TPR proteins revealed that P58IPK TPR 1–3 share significant homol-

ogy with that of the TPR domain of PP5 (fig. 2). In fact, using a computer-assisted structural prediction method, we found a striking structural resemblance between the TPR domain of PP5 and the TPR motifs 1–3 of P58^{IPK} [S. L. Tan and M. G. Katze, unpublished observation]. In this regard, it is worth noting that the crystal structure of the TPR domain of PP5 revealed a homodimer [5], although there is no evidence for TPR-mediated dimerization of full-length PP5 or dimerization of PP5 in solution. Similarly, the oligomeric state of P58^{IPK} in solution has not been carefully examined; thus, we do not yet know which multimeric form(s) of the protein normally exists in vivo. Furthermore, we have not examined whether dimerization is important for P58^{IPK} regulation and function. It is clear from in vitro experiments that TPR motifs 1–3 are not required for interaction with or the inhibition of PKR [36]. However, coexpression of the inactive P58^{IPK}ΔTPR6 protein can exert a dominant-negative effect over wild-type P58^{IPK} protein in inhibiting PKR function in vitro, lending support to the importance of P58^{IPK} dimerization [N. M. Tang, personal communication]. Thus, it will be interesting to determine whether P58^{IPK}ΔTPR6 or other inactive P58^{IPK} mutants can indeed act as dominant-negative mutants, which could serve as valuable reagents in the elucidation of P58^{IPK} function(s).

A search against the *Caenorhabditis elegans* Genome Project Database (http://www.sanger.ac.uk/Projects/C_elegans/blast_server.shtml) revealed a locus, C55B6.2p, encoding a peptide that is 40% identical and 59% similar to human P58^{IPK} (fig. 3). Another P58^{IPK} relative, termed tpr2, was discovered in a yeast two-hybrid screen for proteins that interact with the GAP-related segment of neurofibromin [37]. Tpr2 is 30% identical and 48% similar to bovine P58^{IPK}, containing seven TPR domains and a C-terminal J-domain. Interestingly, the spacing of the TPR domains is conserved between tpr2 and P58^{IPK}, with the exception that there are no counterparts to TPR motifs 4 and 5 of P58^{IPK}. The homology to TPR6 is weak and primarily consists of hydrophobic residues, which probably contribute to the structure of the motif, rather than the interactive surface. Thus, tpr2 will probably not substitute for P58^{IPK} in the regulation of PKR, although this needs to be tested experimentally. At any rate, considering the degeneracy of TPR motifs, the homology between P58^{IPK}, tpr2 and C55B6.2p is remarkable and may suggest an evolutionarily conserved function(s) for this new class of TPR-containing proteins, i.e. those that also contain the classical J-domain.

How does P58^{IPK} regulate PKR pathway: the J connection

Initially, it was thought that P58^{IPK} might function as a pseudosubstrate inhibitor of PKR because the central region of P58^{IPK} has limited homology to the eIF2α, including the conserved serine residue (S51) that is phosphorylated by PKR (fig. 1). However, mutation of this conserved serine residue in P58^{IPK} did not abrogate its ability to inhibit PKR [17]. We propose that P58^{IPK} operates as a cochaperone capable of stimulating Hsp70 via its J-domain to moderate the protein conformation of PKR (fig. 4). As discussed in previous sections, P58^{IPK} is likely held in an inactive complex with Hsp40, possibly along with Hsp70 and PKR, as well as other proteins. In addition to stabilizing P58^{IPK} interaction with Hsp70, Hsp40 may also function as an inhibitor by blocking or competing with P58^{IPK} for stimulating Hsp70. In response to a stress event, such as heat shock or influenza virus infection, Hsp40 dissociates from P58^{IPK} [29], although the mechanism of the release is

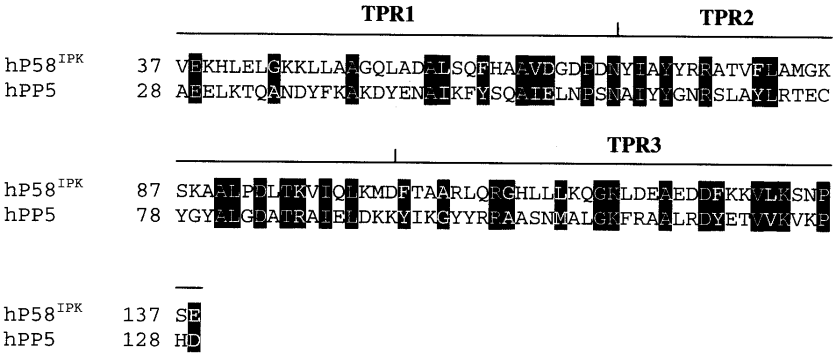

Figure 2. Amino acid sequence comparison between TPR 1–3 of human P58^{IPK} and PP5. Identical residues are indicated by dark boxes; gray boxes denote semiconserved residues.

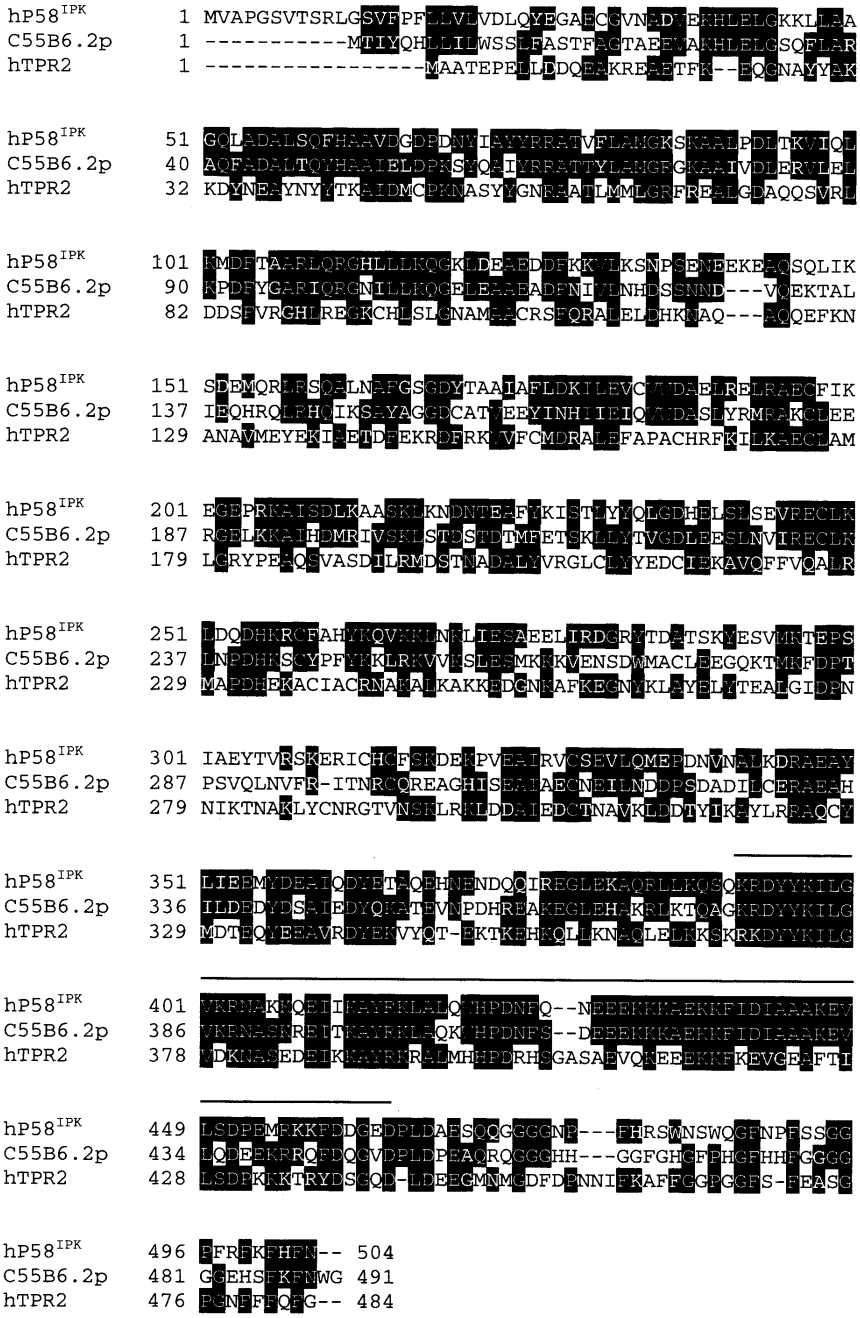

Figure 3. Alignment of human P58IPK, tpr2 and *C. elegans* C55B6.2p. Identical residues are indicated by dark boxes; gray boxes denote semiconserved residues. The J-domains are indicated by top solid bar.

unclear. It is possible that Hsp40 is recruited to protein folding pathways during heat shock (e.g. for refolding denatured proteins) or virus infection (e.g. for viral replication or packaging). Alternatively, Hsp40 may be subjected to some posttranslational modification during a stress event such that it can no longer bind P58IPK. At any rate, Hsp40-free P58IPK is presumably now 'activated' and thus is able to stimulate Hsp70 to inactivate PKR, possibly by inducing a protein conformational change. Perhaps the liberation of Hsp40 alleviates com-

petition between the Hsp40 and P58^{IPK} J-domains for Hsp70 binding, allowing the J-domain of P58^{IPK} to stimulate Hsp70. This suggestion can be tested by measuring the stoichiometry of the proteins before and after heat shock or influenza virus infection.

Consistent with the notion that P58^{IPK} functions as a cochaperone cooperating with Hsp70 to regulate PKR, we demonstrated that P58^{IPK} stimulated the adenosine triphosphatase (ATPase) activity of Hsp70, which promotes the refolding activity of Hsp70 [29]. Presumably this is mediated by the C-terminal J-domain of P58^{IPK}, which is required for the inhibition of PKR in vivo [32]. The J-domain is a conserved region of approximately 70 amino acids that forms three α-helices separated by a loop region, which contains an invariant HPD tripeptide (for recent reviews of J-domain proteins, see [34, 35]). Proteins that contain a J-domain are categorized as members of the DnaJ protein family, although many members share other conserved regions as well. A critical function of the J-domain, such as that of Hsp40, is to regulate the activity of the Hsp70 (DnaK) family of proteins. Hsp40 and Hsp70 work together to promote nascent chain folding of translating proteins, protein transport and secretion [38, 39]. They are also a critical part of the stress response system, helping to prevent the denaturation of proteins during cellular stress and refolding proteins that have been denatured because of cellular stress. The protection and refolding of denatured proteins is achieved by Hsp70 binding directly to the target protein through the C-terminal substrate-binding domain of the chaperone [40]. The substrate-chaperone interaction is stabilized by the hydrolysis of ATP through the N-terminal ATPase domain of Hsp70. It is thought that the prolonged interaction allows more time for the substrate protein to adopt a new conformation, i.e. to become refolded. The role of Hsp40 in this reaction is to stimulate the ATPase activity of Hsp70, which results in improved refolding of the substrate. While the precise mechanism is not known, the stimulation of ATPase activity requires the J-domain of Hsp40 which interacts directly with Hsp70 to stimulate ATPase activity and refolding of denatured substrates [41].

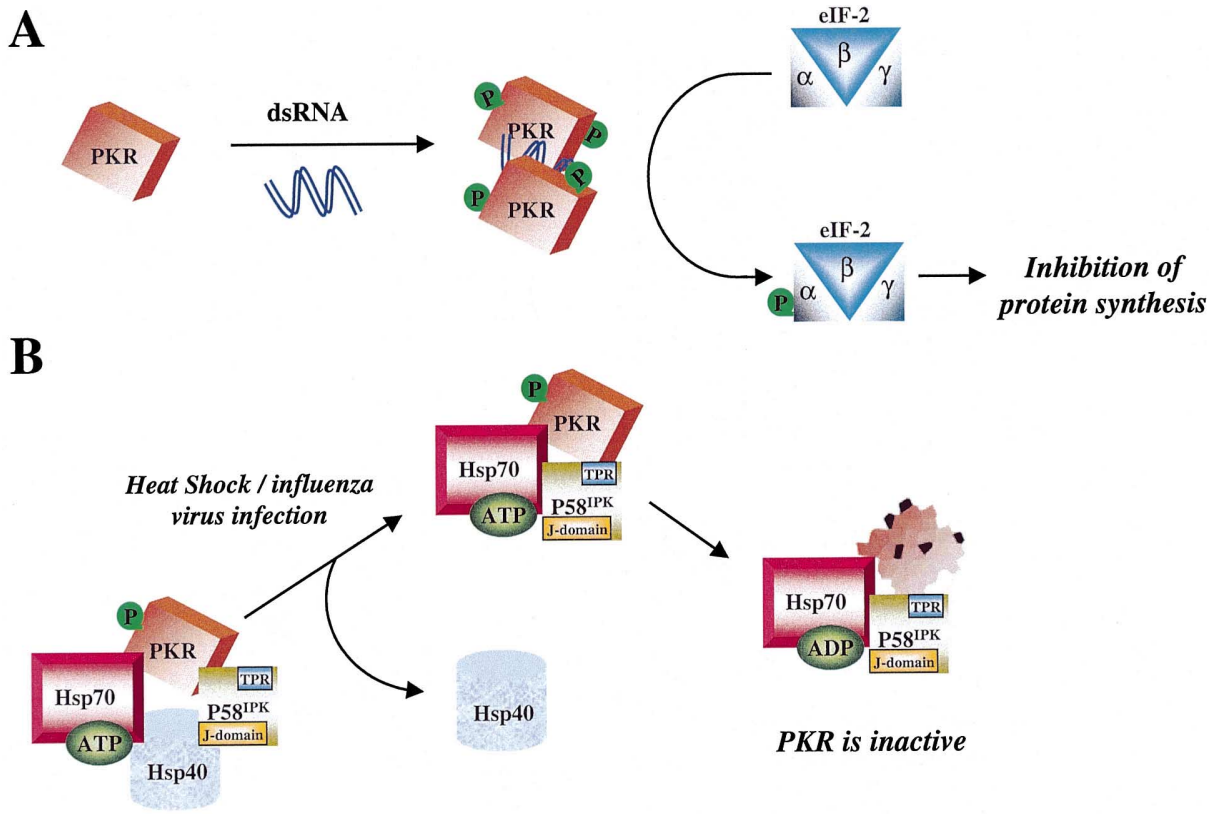

Figure 4. Model for P58^{IPK} regulation of PKR. (*A*) DsRNA-binding to PKR promotes dimerization of PKR, which may be required for optimal kinase activation via transphosphorylation between PKR molecules [61]. (*B*) Proposed co-chaperone function of P58^{IPK} in PKR regulation. See text for further details.

In addition to Hsp40, most of J-domain proteins studied thus far were found to stimulate the ATPase activity of Hsp70 [34, 35].

Recent studies of two other proteins support our model that P58IPK acts as a cochaperone to stimulate Hsp70 to regulate PKR. First is the clathrin-uncoating factor, auxilin, which contains a C-terminal J-domain homology region. The J-domain of auxilin is required for the clathrin uncoating activity, as well as interaction with Hsc70 [42, 43]. The current hypothesis is that auxilin targets Hsc70 to specific sites on the clathrin basket and stimulates the chaperone to refold the clathrin molecule, thus uncoating the vesicle [44]. Indeed, deletion of the J-domain of auxilin abrogates clathrin uncoating [45]. Furthermore, the clathrin-uncoating activity can be blocked by other J-domain proteins, such as yeast Ydj-1 and human Hsj-1 [43, 46]. Presumably, these general chaperones do not contain a clathrin-binding domain, and thus compete with auxilin for Hsc70.

The second protein is the tumor antigen (T-antigen) of the polyomavirus, simian virus 40 (SV40), which has a J-domain at its N-terminus. Work by Kelley and Georgopoulos showed that the T-antigen J-domain could functionally substitute for the J-domain in DnaJ of *Escherichia coli* [47]. More recently, a reciprocal experiment found that the J-domain from Hsj-1 could functionally substitute for the T-antigen J-domain [48]. The T-antigen also contains a binding site for the retinoblastoma tumor suppressor protein (pRb), which the T-antigen may inactivate through direct protein-protein interaction. However, recent reports demonstrate that the J-domain of the T-antigen is critical for the inactivation of pRb and malignant transformation [49, 50]. Srinivasan and colleagues proposed that the T-antigen targets Hsc70 to pRb and stimulates the chaperone to alter the conformation of pRB [49]. This proposition is supported by a report showing that a functional J-domain in the T-antigen is critical for the accumulation of free E2F and activation of E2F promoter sequences [50]. Thus, the T-antigen and auxilin systems highlight the potential of J-domain proteins, such as P58IPK, to target specific proteins to Hsp70. The functional specificity for the different J-domains proteins is conferred by other sequences and structures outside of the J-domain, such as the pRb-binding site for T-antigen.

P58IPK as a potential oncoprotein

P58IPK has recently been demonstrated to possess an antiapoptotic function, protecting cells against TNF-α- and dsRNA-induced cell death [51]. These results are consistent with the idea that P58IPK may be an oncogenic protein; overexpression of P58IPK can lead to malignant transformation in NIH 3T3 cells, which form tumors when injected into nude mice [52]. An inhibition of eIF2α phosphorylation was observed in the transformed cells, suggesting that diminished PKR function in these cells may partly account for the transformed phenotype. Similarly, NIH 3T3 cells whose PKR activity is suppressed by overexpression of catalytically inactive PKR variants also became malignantly transformed and formed tumors in nude mice [52–55].

However, the exact mechanism of the transformation is not known but is thought to involve, at least in part, eIF2α phosphorylation, as overexpression of a nonphosphorylatable form of eIF2α is also transforming [56]. In this regard, messenger RNAs (mRNAs) of many growth factors and protooncogenes contain lengthy 5′ leader sequences, with multiple upstream AUGs and open reading frames [57]. These structures likely serve to inhibit the translation of such mRNAs whose gene products are detrimental to the cell if overproduced. Thus, nonspecific stimulation of translation due to P58IPK inhibition of PKR (hence increased levels of nonphosphorylated eIF2α) may result in increased translation of 'weak' mRNAs encoding proteins that participate in cell growth control or apoptosis.

There is accumulating evidence that PKR is an important effector of apoptotic cell death [58]. Importantly, cells derived from PKR null mice were resistant to apoptosis induced by dsRNA, tumor necrosis factor α (TNF-α) or bacterial lipopolysaccharide (LPS) [59]. Thus, inhibition of PKR may be mechanism by which P58IPK suppresses apoptosis, suggesting another potential mechanism of P58IPK-mediated oncogenesis. In support of this view, NIH 3T3 cells stably expressing P58IPK were refractory to apoptosis induced by TNF-α [51]. However, it remains to be demonstrated that specific mRNAs encoding antiapoptotic proteins and/or protooncogenes are indeed preferentially translated in transformed cells lacking functional PKR (either due to overexpression of P58IPK, PKR transdominant mutants or the nonphosphorylatable eIF2α variant).

In addition to its role in the regulation of PKR activity and mRNA translation, P58IPK may induce oncogenesis through a PKR-independent pathway(s). This possibility arose from our recent observations that NIH 3T3 cell lines expressing a P58IPK mutant that does not bind to or inhibit PKR (P58IPKΔTPR6) also became malignantly transformed [51]. Unlike the case with wild-type P58IPK cell lines [52], P58IPKΔTPR6 cell lines did not display any significant reduction in PKR activity or eIF2α phosphorylation [51]. Furthermore, both the P58IPK and P58IPKΔTPR6 cell lines were refractory to apoptosis induced by TNF-α. However, consistent with its role as a PKR inhibitor, overexpression of P58IPK, but not P58IPKΔTPR6, did result in increased resistance to dsRNA-induced apoptosis. Thus, as expected of the complex and multifactorial process of tumorigenesis,

the oncogenic properties of $P58^{IPK}$ are likely due to both the suppression of PKR-mediated eIF2α phosphorylation and the activation of one or more tumorigenic pathways. One possible mechanism may involve the nuclear transcription factor-kappa β (NFκB) pathway, as it was found that $P58^{IPK}$ or $P58^{IPK}\Delta$TPR6 overexpression could lead to constitutive activation of the antiapoptotic effector NFκB [51]. In this regard, constitutive activation of NFκB and subsequent suppression of apoptosis was recently associated with Ras-induced malignant transformation [60, 61].

A potential clue that $P58^{IPK}$ can induce malignant transformation via yet another different mechanism may lie in the C-terminal J-domain of the protein, which has been shown to mediate oncogenesis in other systems. As described in the previous section, the presence of J-domain in the T-antigen of SV40 (TAg) is required for virus-induced oncogenesis [48–50]. The J-domain of TAg and other polyomavirus large T-antigens is also necessary for binding to and inactivation of pRB [48]. Importantly, the J-domain of TAg is capable of stimulating the ATPase activity of members of the Hsp70 family of proteins [49]. These observations have led to the hypothesis that SV40 transforms cells at least in part through the J-domain of TAg, which functions to direct Hsp70 to the E2F-pRB complex [50]. This results in the release of active E2F transcription factor and the subsequent expression of genes, which in turn promote entry of cells into S-phase. A similar scenario may be envisioned for $P58^{IPK}$ whereby the J-domain of the protein directs Hsp70 to inactivate a tumor suppressor protein, which may be putative PKR tumor suppressor or other unidentified $P58^{IPK}$-interacting proteins, leading to the induction of oncogenesis. Finally, the possibility that other TPR motifs of $P58^{IPK}$, each of which could potentially mediate with other cellular proteins, may play a role in tumorigenesis cannot be excluded.

Future directions and concluding remarks

The $P58^{IPK}$-PKR pathway provides an excellent model system to study TPR motifs and the ways in which they distinguish between different substrates. TPR6 and TPR7 interact with PKR and $P52^{rIPK}$, respectively, but the residues that confer specificity to the interactions are not known. Candidate residues that may contact PKR and $P52^{rIPK}$ directly can be determined by generating point mutants that disrupt $P58^{IPK}$ binding to PKR and/or $P52^{rIPK}$. The location of the residues within the TPR structure will likely be conserved from motif to motif; there is probably a subset of amino acids within a TPR motif that confer specificity. By identifying those residues in $P58^{IPK}$, we may be able to define similar determinants in other TPR proteins. Obviously, determining the crystal structure of $P58^{IPK}$ complexed with PKR or $P52^{rIPK}$ would also provide important insights into these questions. Similarly, an alignment of different TPR-interacting proteins may suggest possible defining residues for TPR recognition.

It will be important to confirm the role of Hsp70 in the regulation of PKR. A $P58^{IPK}$ variant mutated in the conserved HPD tripeptide of the J-domain should not be able to stimulate the ATPase activity of Hsp70 and inhibit PKR. In addition, the mechanism of the release of $P58^{IPK}$ by Hsp40 in influenza virus-infected cells needs to be addressed. It may be that Hsp40 is recruited to another site in the cell, or that it is modified in some way. Curiously, this dissociation was also observed in cells recovering from heat shock [29]. It is tempting to speculate that $P58^{IPK}$ activation may be part of a typical stress response, and influenza virus infection may simply trigger this response. Indeed, influenza virus infection of lung tissue mimics the cellular stress induced by oxidative stress [62]. Similarly, it will be important to identify the conditions under which $P52^{rIPK}$ and $P58^{IPK}$ interact within the cell, as well as the consequences of this interaction in vivo. Perhaps once we know more about the posttranslational regulation of Hsp40 and $P52^{rIPK}$ in stressed cells, we will gain a better understanding of how Hsp40 modulates $P58^{IPK}$.

We would also like to know how Hsp70 might recognize PKR as a substrate in the context of bound $P58^{IPK}$. The answer may be provided by the recent observation that $P58^{IPK}$ blocked dimer formation of PKR [63]. Monomerization of PKR by $P58^{IPK}$ may expose hydrophobic domains on PKR to Hsp70, which binds and subsequently refolds the kinase. Interestingly, monomerization of the *E. coli* P1 replication initiator RepA requires a functional DnaK-DnaJ system [64, 65], thus implicating involvement of a J-domain member in dissociating of protein complex. Refolding by Hsp70 may render PKR in a defective conformation in binding activator dsRNA, kinase activity, substrate recognition or dimerization. One can begin to answer these questions by comparing the structural conformation of PKR in the presence of Hsp70 with either wild-type $P58^{IPK}$ or a $P58^{IPK}$ protein containing a mutation in the HPD tripeptide by using protease digestion mapping.

The $P58^{IPK}$ study and others suggest an emerging theme in the molecular chaperone field – molecular chaperones are recruited by distinct cochaperones to specific pathways. The Cdc37 system is another example of a cochaperone that specifically modulates the activity of a subset of protein kinases through its ability to interact with molecular chaperones [66, 67]. Cdc37p binds to kinases such as Cdk4, Ras and v-Src in association with Hsp90, and is required for the maturation of the kinase to a fully functional form. Similarly, the activation of steroid receptor is regulated by protein-protein interac-

tions between molecular chaperones Hsp40, Hsp70 and Hsp90 with cochaperones, including Hip, Hop, Cdc37, Cyp-40 and the FKBPs [8, 68]. P58^{IPK} may represent a novel class of cochaperones in that it is capable of promoting simultaneous interactions with other molecular chaperones through both its TPR motifs and J-domain to refold and inactivate PKR activity. It would be interesting to examine whether Hsp90 and/or other cochaperones are also part of a larger molecular chaperone complex with P58^{IPK} that modulates PKR activity, as well as other protein kinases.The oncogenic potential of P58^{IPK} is interesting, as members of molecular chaperones have been associated with a variety of disease states, including cancer [69, 70]. P58^{IPK}-induced malignant transformation is likely to be a complex phenomenon in which multiple pathways are simultaneously activated through the various functional domains of the protein. Some of these pathways may involve molecular chaperones through the recruitment power of the J-domain of P58^{IPK}, whereas others may involve PKR or other unidentified proteins recruited by P58^{IPK} TPR motifs. At present, it appears that P58^{IPK}-induced oncogenesis involves the molecular intersection between IFN and stress response, cell death and growth-regulatory pathways.

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1 Goebl M. and Yanagida M. (1991) The TPR snap helix: a novel protein repeat motif from mitosis to transcription. Trends Biochem. Sci. **16:** 173–177
2 Lamb J. R., Tugendreich S. and Hieter P. (1995) Tetratricopeptide repeat interactions: to TPR or not to TPR? Trends Biochem. Sci. **20:** 257–259
3 Hirano T., Kinoshita N., Morikawa K. and Yanagida M. (1990) Snap helix with knob and hole: essential repeats in *S. pombe* nuclear protein nuc2+. Cell **60:** 319–328
4 Sikorski R. S., Boguski M. S., Goebl M. and Hieter P. (1990) A repeating amino acid motif in *CDC23* defines a family of proteins and a new relationship among genes required for mitosis and RNA synthesis. Cell **60:** 307–317
5 Das A. K., Cohen P. W. and Barford D. (1998) The structure of the tetratricopeptide repeats of protein phosphatase 5: implications for TPR-mediated protein-protein interactions. EMBO J. **17:** 1192–1199
6 Lamb J. R., Michaud W. A., Sikorski R. S. and Hieter P. A. (1994) Cdc16p, Cdc23p and Cdc27p form a complex essential for mitosis. EMBO J. **13:** 4321–4328
7 Tzamarias D. and Struhl K. (1995) Distinct TPR motifs of Cyc8 are involved in recruiting the Cyc8-Tup1 corepressor complex to differentially regulated promoters. Genes Dev. **9:** 821–831
8 Smith D. F. (1998) Sequence motifs shared between chaperone components participating in the assembly of progesterone receptor complexes. Biol. Chem. **379:** 283–288
9 Owens-Grillo J. K., Hoffmann K., Hutchison K. A., Yem A. W., Deibel M. R. Jr, Handschumacher R. E. et al. (1995) The cyclosporin A-binding immunophilin CyP-40 and the FK506-binding immunophilin hsp56 bind to a common site on hsp90 and exist in independent cytosolic heterocomplexes with the untransformed glucocorticoid receptor. J. Biol. Chem. **270:** 20479–20484
10 Chen S., Prapapanich V., Rimerman R., Honore B. and Smith D. (1996) Interactions of p60, a mediator of progesterone receptor assembly, with heat shock proteins hsp90 and hsp70. Mol. Endocrinol. **10:** 682–693
11 Chen M.-S., Silverstein A. M., Pratt W. B. and Chinkers M. (1996) The tetratricopeptide repeat domain of protein phosphatase 5 mediates binding to glucocorticoid receptor heterocomplexes and acts as a dominant negative mutant. J. Biol. Chem. **50:** 32315–32320
12 Carello A., Ingley E., Minchin R. F., Schickwann T. and Ratajczak T. (1999) The common tetratricopeptide repeat acceptor site for steroid receptor-associated immunophilins and Hop is located in the dimerization domain of Hsp90. J. Biol. Chem. **274:** 2682–2689
13 Irmer H. and Höhfeld J. (1997) Characterization of functional domains of the eukaryotic co-chaperone Hip. J. Biol. Chem. **272:** 2230–2235
14 Ballinger C. A., Connell P., Wu Y. X., Hu Z. Y., Thompson L. J., Yin L. Y. et al. (1999) Identification of CHIP, a novel tetratricopeptide repeat-containing protein that interacts with heat shock proteins and negatively regulates chaperone functions. Mol. Cell. Biol. **19:** 4535–4545
15 Prapapanich V., Chen S., Nair S. C., Rimerman R. A. and Smith D. F. (1996) Molecular cloning of human p48, a transient component of progesterone receptor complexes and an Hsp70-binding protein. Mol. Endocrinol. **10:** 420–431
16 Lee T. G., Tomita J., Hovanessian A. G. and Katze M. G. (1990) Purification and partial characterization of a cellular inhibitor of the interferon-induced protein kinase of M_r 68,000 from influenza virus-infected cells. Proc. Natl. Acad. Sci. USA **87:** 6208–6212
17 Lee T. G., Tang N., Thompson S., Miller J. and Katze M. G. (1994) The 58,000-dalton cellular inhibitor of the interferon-induced double-stranded RNA-activated protein kinase (PKR) is a member of the tetratricopeptide repeat family of proteins. Mol. Cell. Biol. **14:** 2331–2342
18 Clemens M. J. and Elia A. (1997) The double-stranded RNA-dependent protein kinase PKR: structure and function. J. Interferon Cytokine Res. **17:** 503–524
19 Gale M. Jr and Katze M. G. (1998) Molecular mechanisms of interferon resistance mediated by viral-directed inhibition of PKR, the interferon-induced protein kinase. Pharmacol. Ther. **78:** 29–46
20 Chang H.-W., Watson J. C. and Jacobs B. L. (1992) The E3L gene of vaccinia virus encodes an inhibitor of the interferon-induced, double-stranded RNA-dependent protein kinase. Proc. Natl. Acad. Sci. USA **89:** 4825–4829
21 Langland J. O., Pettiford S., Jiang B. and Jacobs B. L. (1994) Products of the porcine group C rotavirus NSP3 gene bind specifically to double-stranded RNA and inhibit activation of the interferon-induced protein kinase PKR. J. Virol. **68:** 3821–3829
22 Schneider R. J. (1996) Adenovirus and vaccinia virus translational control. In: Translational Control, vol. 1, pp. 575–606, Hershey J., Mathews M. B. and Sonenberg N. (eds), Cold Spring Harbor Press, Cold Spring Harbor, NY
23 Davies M. V., Elroy-Stein O., Jagus R., Moss B. and Kaufman R. J. (1992) The vaccinia virus K3L gene product potentiates translation by inhibiting double-stranded-RNA-activated protein kinase and phosphorylation of the alpha subunit of eukaryotic initiation factor 2. J. Virol. **66:** 1943–1950

24 Brand S. R., Kobayashi R. and Mathews M. B. (1997) The Tat protein of human immunodeficiency virus type 1 is a substrate and inhibitor of the interferon-induced, virally activated protein kinase, PKR. J. Biol. Chem. **272:** 8388–8395

25 Gale M. J. Jr, Korth M. J., Tang N. M., Tan S.-L., Hopkins D. A., Dever T. E. et al. (1997) Evidence that hepatitis C virus resistance to interferon is mediated through repression of the PKR protein kinase by the nonstructural 5A protein. Virology **230:** 217–227

26 Gale M. J. Jr, Blakely C. M., Kwieciszewski B., Tan S.-L., Dossett M., Tang N. M. et al. (1998) Control of PKR protein kinase by hepatitis C virus nonstructural 5A protein: molecular mechanisms of kinase regulation. Mol. Cell. Biol. **18:** 5208–5218

27 Black T. L., Barber G. N. and Katze M. G. (1993) Degradation of the interferon-induced 68,000-M_r protein kinase by poliovirus requires RNA. J. Virol. **67:** 791–800

28 Gale M. Jr, Blakely C. M., Hopkins D. A., Melville, M. W., Wambach M., Romano P. R. et al. (1998) Regulation of interferon-induced protein kinase PKR: modulation of $P58^{IPK}$ inhibitory function by a novel protein, $P52^{rIPK}$. Mol. Cell. Biol. **18:** 859–871

29 Melville M. W., Hansen W. J., Freeman B. C., Welch W. J. and Katze M. G. (1997) The molecular chaperone hsp40 regulates the activity of $P58^{IPK}$, the cellular inhibitor of PKR. Proc. Natl. Acad. Sci. USA **94:** 97–102

30 Melville M. W., Tan S.-L., Wambach M., Song J., Morimoto R. I. and Katze M. G. (1999) The cellular inhibitor of the PKR protein kinase, $P58^{IPK}$, is an influenza virus-activated co-chaperone that modulates heat shock protein 70 activity. J. Biol. Chem. **274:** 3797–3803

31 Gale M. Jr, Tan S.-L., Wambach M. and Katze M. G. (1996) Interaction of the interferon-induced PKR protein kinase with inhibitory proteins $P58^{IPK}$ and vaccinia virus K3L is mediated by unique domains: implications for kinase regulation. Mol. Cell. Biol. **16:** 4172–4181

32 Tang N. M., Ho C. Y. and Katze M. G. (1996) The 58-kDa cellular inhibitor of the double stranded RNA-dependent protein kinase requires the tetratricopeptide repeat 6 and DnaJ motifs to stimulate protein synthesis in vivo. J. Biol. Chem. **271:** 28660–28666

33 Frydman J. and Hohfeld J. (1997) Chaperones get in touch: the Hip-Hop connection. Trends Biochem. Sci. **22:** 87–92

34 Cheetham M. E. and Caplan A. J. (1998) Structure, function and evolution of DnaJ: conservation and adaptation of chaperone function. Cell Stress and Chaperones **3:** 28–36

35 Kelley W. L. (1998) The J-domain family and the recruitment of chaperone power. Trends Biochem. Sci. **23:** 222–227

36 Polyak S. J., Tang N., Wambach M., Barber G. N. and Katze M. G. (1996) The P58 cellular inhibitor complexes with the interferon-induced, double-stranded RNA-dependent protein kinase, PKR, to regulate its autophosphorylation and activity. J. Biol. Chem. **271:** 1702–1707

37 Murthy A. E., Bernards A., Church D., Wasmuth J. and Gusella J. F. (1996) Identification and characterization of two novel tetratricopeptide repeat-containing genes. DNA and Cell Biol. **15:** 727–735

38 Beissinger M. and Buchner J. (1998) How chaperones fold proteins. Biol. Chem. **379:** 245–259

39 Hartl F. U. (1996) Molecular chaperones in cellular protein folding. Nature **381:** 571–580

40 Hightower L. E., Sadis S. E. and Takenaka I. M. (1994) Interactions of vertebrate hsc70 and hsp70 with unfolded proteins and peptides. In: The Biology of Heat Shock Proteins and Molecular Chaperones, vol. 1, pp. 179–208, Morimoto R. I., Tissres A. and Georgopoulos C. (eds), Cold Spring Harbor Press, Cold Spring Harbor, New York

41 Tsai J. and Douglas M. G. (1996) A conserved HPD sequence of the J-domain is necessary for YDJ1 stimulation of Hsp70 ATPase activity at a site distinct from substrate binding. J. Biol. Chem. **271:** 9347–9354

42 Jiang R.-F., Greener T., Barouch W., Greene L. and Eisenberg E. (1998) Interaction of auxilin with the molecular chaperone, Hsc70. J. Biol. Chem. **272:** 6141–6145

43 King C., Eisenberg E. and Greene L. (1997) Effect of yeast and human DnaJ homologs on clathrin uncoating by 70 kilodalton heat shock protein. Biochemistry **36:** 4067–4073

44 Ungewickell E., Ungewickell H. and Holstein S. E. H. (1997) Functional interaction of the auxilin J domain with the nucleotide- and substrate-binding modules of Hsc70. J. Biol. Chem. **272:** 19594–19600

45 Ungewickell E. et al. (1995) Role of auxilin in uncoating clathrin-coated vesicles. Nature **378:** 632–635

46 Cheetham M. E., Anderton B. H. and Jackson A. P. (1996) Inhibition of hsc70-catalysed clathrin uncoating by HSJ1 proteins. Biochem. J. **319:** 103–108

47 Kelley W. L. and Georgopoulos C. (1997) The T/t common exon of simian virus 40, JC and BK polyomavirus T antigens can functionally replace the J-domain of the *Escherichia coli* DnaJ molecular chaperone. Proc. Natl. Acad. Sci. USA **94:** 3679–3684

48 Zalvide J., Stubdal H. and DeCaprio J. A. (1998) The J domain of simian virus 40 large T antigen is required to functionally inactivate RB family proteins. Mol. Cell. Biol. **18:** 1408–1415

49 Srinivasan A., McClellan A. J., Vartikar J., Marks I., Cantalupo P., Li Y. et al. (1997) The amino-terminal transforming region of simian virus 40 large T and small t antigens functions as a J domain. Mol. Cell. Biol. **17:** 4761–4773

50 Harris K. F., Christensen J. B., Radany E. H. and Imperiale M. J. (1998) Novel mechanisms of E2F induction by BK virus large-T antigen: requirement of both the pRb-binding and the J domains. Mol. Cell. Biol. **18:** 1746–1756

51 Tang N. M., Korth M. J., Gale M. Jr, Wambach M., Der S. D., Bandayopadhyay S. K. et al. (1999) Inhibition of double-stranded RNA- and tumor necrosis factor alpha-mediated apoptosis by tetratricopeptide repeat protein and cochaperone $P58^{IPK}$. Mol. Cell. Biol. **19**: 4757–4765.

52 Barber G. N., Thompson S., Lee T. G., Strom T., Jagus R. et al. (1994) The 58-kilodalton inhibitor of the interferon-induced double-stranded RNA-activated protein kinase is a tetratricopeptide repeat protein with oncogenic potential. Proc. Natl. Acad. Sci. USA **91:** 4278–4282

53 Koromilas A. E., Roy S., Barber G. N., Katze M. G. and Sonenberg N. (1992) Malignant transformation by a mutant of the IFN-inducible dsRNA-dependent protein kinase. Science **257:** 1685–1689

54 Meurs E., Galabru J., Barber G. N., Katze M. G. and Hovanessian A. G. (1993) Tumor suppressor function of the interferon-induced double-stranded RNA-activated protein kinase. Proc. Natl. Acad. Sci. USA **90:** 232–236

55 Barber G. N., Wambach M., Thompson S., Jagus R. and Katze M. G. (1995) Mutants of the RNA-dependent protein kinase (PKR) lacking double-stranded RNA binding domain I can act as transdominant inhibitors and induce malignant transformation. Mol. Cell. Biol. **15:** 3138–3146

56 Donzé O., Jagus R., Koromilas A. E., Hershey J. W. B. and Sonenberg N. (1995) Abrogation of translation initiation factor eIF-2 phosphorylation causes malignant transformation of NIH 3T3 cells. EMBO J. **14:** 3828–3834

57 Korth, M. J. and Katze M. G. (1997) mRNA metabolism and cancer. In: mRNA Metabolism and Post-transcriptional Gene Regulation, vol. 1, pp. 265–280, Harford J. B. and Morris D. R. (eds), Wiley-Liss, New York

58 Tan S.-L. and Katze M. G. (1999) The emerging role of the interferon-induced PKR protein kinase as an apoptotic effector: a new face of death? J. Interferon Cytokine Res. **19:** 545–556

59 Der S. D., Yang Y.-L., Weissman C. and Williams B. R. G. (1997) A double-stranded RNA-activated protein kinase-dependent pathway mediating stress-induced apoptosis. Proc. Natl. Acad. Sci. USA **94:** 3279–3283

60 Finco T. S., Westwick J. K., Norris J. L., Beg, A. A., Der C. J. and Baldwin A. S. Jr (1997) Oncogenic Ha-Ras-induced

signaling activates NF-kappaB transcriptional activity, which is required for cellular transformation. J. Biol. Chem. **272**: 24113–24116.
61 Mayo M. W., Wang C.-Y., Cogswell P. C., Rogers Graham K. S., Lowe S. W., Der C. J. et al. (1997) Requirement of NF-kappaB activation to suppress p53-independent apoptosis induced by oncogenic Ras. Science **278:** 1812–1815
62 Choi A. M., Knobil K., Otterbein S. L., Eastman D. A. and Jacoby D. B. (1996) Oxidant stress responses in influenza virus pneumonia: gene expression and transcription factor activation. Am. J. Physiol. **271 (3 Pt 1):** 383–391
63 Tan S.-L., Gale M. Jr and Katze M. G. (1998) Double-stranded RNA-independent dimerization of interferon-induced protein kinase PKR and inhibition of dimerization by the cellular P58^{IPK} inhibitor. Mol. Cell. Biol. **18:** 2431–2443
64 Wickner S., Hoskins J. and McKenney K. (1991) Monomerization of RepA dimers by heat shock proteins activates binding to DNA replication origin. Proc. Natl. Acad. Sci. USA **88:** 7903–7907
65Wickner S., Skowyra D., Hoskins J. and McKenney K. (1992) DnaJ, DnaK and GrpE heat shock proteins are required in oriP1 DNA replication solely at the RepA monomerization step. Proc. Natl. Acad. Sci. USA **89:** 10249–10345
66 Kimura Y., Rutherford S. L., Miyata Y., Yahara I., Freeman B. C., Yue L. et al. (1997) Cdc37 is a molecular chaperone with specific functions in signal transduction. Genes Dev. **11:** 1775–1785
67 Hunter T. (1997) Cdc37: a protein kinase chaperone? Trends Cell Biol. **7:** 157–161
68 Pratt W. B. and Toft D. O. (1997) Steroid receptor interactions with heat shock protein and immunophilin chaperones. Endocr. Rev. **18:** 306–360
69 Morimoto R. I., Kline M. P., Bimston D. N. and Cotto J. J. (1997) The heat-shock response: regulation and function of heat-shock proteins and molecular chaperones. Essays Biochem. **32:** 17–29
70 Soti C. and Csermely P. (1998) Molecular chaperones in the etiology and therapy of cancer. Pathol. Oncol. Res. **4:** 316–321
71 Dolinski K. J., Cardenas M. E. and Heitman J. (1998) CNS1 endodes an essential p60/Sti1 homolog in *Saccharomyces cerevisiae* that suppresses cyclophilin 40 mutations and interacts with Hsp90. Mol. Cell. Biol. **18:** 7344–7352
72 Duina A. A., Chang H.-C. J., Marsh J. A., Lindquist S. and Gaber R. F. (1996) A cyclophilin function in Hsp90-dependent signal transduction. Science **274:** 1713–1715
73 Duina A. A., Marsh J. A. and Gaber R. F. (1996) Identification of two Cyp-40-like cyclophilins in *Saccharomyces cerevisiae*, one of which is required for normal growth. Yeast **12:** 943–952
74 Warth R., Briand P.-A. and Picard D. (1997) Functional analysis of the yeast 40 kDa cyclophilin Cyp40 and its role for viability and steroid receptor regulation. Biol. Chem. **378:** 381–391
75 Brocard C., Kragler F., Simon M. M., Schuster T. and Hartig A. (1994) The tetratricopeptide repeat-domain of the PAS10 protein of Saccharomyces cerevisiae is essential for binding the peroxisomal targeting signal — SKL. Biochem. Biophys. Res. Comm. **204:** 1016–1022
76 Lockhart S. R. and Rymond B. C. (1994) Commitment of yeast pre-mRNA to the splicing pathway requires a novel small nuclear ribonucleoprotein polypeptide, Prp39p. Mol. Cell. Biol. **14:** 3623–3633
77 Fromont-Racine M., Rain J. C. and Legrain P. (1997) Toward a functional analysis of the yeast genome through exhaustive two-hybrid screens. Nature Genet. **16:** 277–282
78 McLean M. R. and Rymond B. C. (1998) Yeast pre-mRNA splicing requires a pair of U1 snRNP-associated tetratricopeptide repeat proteins. Mol. Cell. Biol. **18:** 353–360
79 Chang H.-C. J., Nathan D. F. and Lindquist S. (1997) In vivo analysis of the Hsp90 cochaperone Sti1 (p60). Mol. Cell. Biol. **17:** 318–325
80 McCollum D., Monosov E. and Subramani S. (1993) The pas8 mutant of Pichia pastoris exhibits the peroxisomal protein import deficiencies of Zellweger syndrome cells – the PAS8 protein binds to the COOH-terminal tripeptide peroxisomal targeting signal, and is a member of the TPR protein family. J. Cell Biol. **121:** 761–774
81 Mirabito P. M. and Morris N. R. (1993) BIMA, a TPR-containing protein required for mitosis, localizes to the spindle pole body in *Aspergillus nidulans*. J. Cell Biol. **120:** 959–968
82 Cali B. M., Kuchma S. L., Latham J. and Anderson P. (1999) *smg-7* is required for mRNA surveillance in *Caenorhabditis elegans*. Genetics **151:** 605–616
83 Gindhart J. G. Jr and Goldstein L. S. B. (1996) Tetratrico peptide (sic) repeats are present in the kinesin light chain. Trends Biochem. Sci. **21:** 52–53
84 Owens-Grillo J. K., Stancato L. F., Hoffmann K., Pratt W. B. and Krishna P. (1996) Binding of immunophilins to the 90 kDa heat shock protein (hsp90) via a tetratricopeptide repeat domain is a conserved protein interaction in plants. Biochem. **35:** 15249–15255
85 Ollendorff V. and Donoghue D. J. (1997) The serine/threonine phosphatase PP5 interacts with CDC16 and CDC27, two tetratricopeptide repeat-containing subunits of the anaphase-promoting complex. J. Biol. Chem. **272:** 32011–32018
86 Nair S. C., Rimerman R. A., Toran E. J., Chen S., Prapapanich V., Butts R. N. et al. (1997) Molecular cloning of human FKBP51 and comparisons of immunophilin interactions with Hsp90 and progesterone receptor. Mol. Cell. Biol. **17:** 594–603
87 Radanyi C., Chambraud B. and Baulieu E.-E. (1994) The ability of the immunophilin FKBP59-HBI to interact with the 90-kDa heat shock protein is encoded by its tetratricopeptide repeat domain. Proc. Natl. Acad. Sci. USA **91:** 11197–11201
88 Höhfeld J., Miname Y. and Hartl F.-U. (1995) Hip, a novel cochaperone involved in the eukaryotic Hsc70/Hsp40 reaction cycle. Cell **83:** 589–4598